Using modern reproductive methods to hybridize Old and New World Camelids: *Camelus dromedarius* x *Lama guanicoe*

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Key words

Camelus dromedarius - *Lama guanicoe* - Hybridization - Artificial insemination - United Arab Emirates.

Summary

A group of nine female and one male guanaco were maintained alongside a larger herd of dromedary camels at the Camel Reproduction Centre in Dubai. The ovaries of the female guanacos and camels were scanned regularly, and when they exhibited a dominant follicle of 0.7-0.9 cm diameter in the guanaco, or 1.3-1.8 cm diameter in the dromedaries, they were given a single injection of the GnRH analogue buserelin (Receptal; camels 20 µg i.v., guanacos 10 µg i.m.) to induce ovulation and were inseminated 24 h later. Thirty female dromedaries were inseminated on 50 occasions with 2-4 ml of guanaco semen diluted 1:1 with green buffer (150-400 x 10^6 motile spermatozoa), producing two pregnancies; one resulted in a stillborn on day 365 of gestation, the other was aborted on day 260. Similarly, nine female guanacos were inseminated on 34 occasions with diluted dromedary semen (300-400 x 10^6 motile spermatozoa), which resulted in six conceptions; two were resorbed between days 25-40 after ovulation, two were aborted on days 291 and 302, respectively, one was stillborn on day 365, and one male calf was born live on day 328. To the knowledge of the authors, this is the first ever viable hybrid between Old and New World Camelids. Its existence highlights a remarkable degree of conservation of reproductive processes between them.

■ INTRODUCTION

The family Camelidae is of great antiquity. Paleontological evidence suggests they split off from the other cloven-hoofed mammals in the Eocene around 40-45 million years ago (10, 13), and split again into the genera *Camelus* and *Lama* relatively soon thereafter, around 30 million years ago (8, 21). However, more recent studies of mitochondrial DNA mutation rates suggest that the split may have occurred around 11 million years ago (19).

During one of the early ice ages some of the cameloids migrated across the Bering Straits, and thence radiated through Asia into Eastern Europe, the Middle East and North Africa, to develop into the present day Bactrian (*Camelus bactrianus*) and dromedary (*Camelus dromedarius*) camels (Old World Camelids), whereas the lamoids migrated southwards and crossed the land bridge into South America and evolved into the New World or South American Camelids namely the domesticated llama (*Lama glama*), its probable wild antecedent the guanaco (*Lama guanicoe*), the domesticated alpaca (*Lama pacos*) and its possible wild ancestor the vicuña (*Lama vicugna*).

Today, New and Old World Camelids show some anatomical and physiological similarities, but some equally striking contrasts. For example, they all share the same chromosome number (2n = 74; ref. 9), they all exhibit the same disproportion of the bicornuate uterus, whereby the left horn is considerably larger than the right

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and is always the site of implantation (4), and they also share the same diffuse, non-invasive epitheliochorial placenta (18, 20). In contrast, however, New World Camelids are small, cloven-hoofed, and have a dense, fine wool coat which enables them to survive at the low temperatures of the deserts of the high Andes, whereas Old World Camelids are large, slow, have a single footpad and cope ideally with extreme temperatures and harsh food availability of the low-lying deserts of Arabia and Africa. Both Old World Camelids will hybridize with one another to produce fertile offspring (5), and all New World species will similarly hybridize with each other and produce fertile offspring (5). However, there are no published accounts of successful hybridization between Old and New World species, which would not occur naturally due to their different geographical locations and the marked differences in body size. The object of the present experiment was to attempt to hybridize dromedaries with guanacos, using artificial insemination to overcome the 6-fold size difference between parental species.

■ MATERIALS AND METHODS

Thirty adult female camels aged 6-14 years and weighing between 380 and 450 kg and two male dromedaries aged 5 and 8 years, of estimated weight 550-600 kg, that had been trained to ejaculate into a modified bull artificial vagina (AV) were maintained as part of the experimental herd at the Camel Reproduction Centre in Dubai. Nine female (estimated age 3-7 years) and one young male (estimated age 3-4 years) guanacos that had been maintained on a nearby wildlife park were captured and translocated to pens at the Camel Reproduction Centre where the male was trained to ejaculate into a smaller modified ram AV.

During the camel-breeding season in the Gulf region (November-April) the ovarian follicular wave patterns of the dromedaries were monitored regularly by serial transrectal ultrasound examinations as described by Skidmore *et al.* (15). When a dominant follicle reached 1.3-1.6 cm in diameter the camel was given an intravenous (i.v.) injection of 20 µg of the GnRH analogue buserelin (Receptal; Hoechst Animal Health, Beds., UK) and inseminated with the whole ejaculate from the male guanaco, (2-4 ml; motility 50-70%; concentration 150-400 x 10^6 spermatozoa) collected by AV, and diluted with an equal volume of the green buffer (IMV) with 10% v:v added egg yolk. The semen was deposited into the uterus by means of a manually guided insemination catheter passed through the cervix and these inseminations occurred either once, 24 h after treatment with GnRH (n = 45), or twice, at the time of GnRH therapy and again 24 h later (n = 5).

Similarly, the ovarian follicular wave patterns in the female guanacos were monitored by transrectal ultrasonography and when the dominant follicle attained a diameter of 0.8-0.9 cm the guanaco was given an intramuscular (i.m.) injection of 10 µg buserelin and inseminated either once only 24 h later (n = 22) with 4-6ml of camel semen (300-400 x 10^6 spermatozoa, motility 60-80%) diluted 1:1 with green buffer containing 10% v:v egg yolk, or twice, at the time of GnRH treatment and 24 h later (n = 11). It was inseminated as described previously.

Ovulation was diagnosed by ultrasound examination of the ovaries 48 h after GnRH injection (15) and confirmed subsequently by measuring a rise in progesterone concentrations in peripheral serum samples recovered daily from each animal from the time of GnRH treatment. A chemiluminescent progesterone assay method developed for human serum (Amerlite; Kodak Diagnostics, Bucks., UK) and validated for camel serum by Skidmore *et al.* was used (16). Pregnancy was suspected when serum progesterone concentrations remained elevated beyond 12 days after insemination and it was confirmed subsequently by ultrasound examination of the uterus from day 18 onwards (14).

However, in two of the inseminated camels the luteal function was suspected of being impaired as judged by the ultrasonographic appearance of the *corpus lutea*. These animals were therefore given daily i.m. injections of 150 mg progesterone-in-oil (Intervet Laboratories, Cambridge, UK) from day 6 after insemination until one of them was confirmed non-pregnant by ultrasound examination on days 22 and 25 and the other aborted spontaneously at 9 months of gestation.

■ RESULTS

Thirty female dromedaries were inseminated on 50 occasions with diluted guanaco semen which resulted in two pregnancies; one was stillborn on day 365 of gestation and one aborted a dead and partly autolyzed female fetus on day 260 (table I). Similarly, nine female guanacos were inseminated on 34 occasions with diluted dromedary semen which resulted in six conceptions; two were resorbed between days 25-40 after ovulation, two were aborted on days 291 and 302, one was stillborn on day 365. The sixth guanaco calved spontaneously and unaided on day 328 of gestation and produced a live male calf, that was somewhat premature and weighed only 5.5 kg. This is less than the weight of a newborn guanaco at term (8-10 kg) and much less than the weight of a newborn camel calf (± 30 kg).

The calf showed no tooth eruption at birth and, since its mother had no mammary development and showed no maternal behavior, the hybrid calf was hand-reared on fresh camel milk, recovered daily from a female dromedary that had calved 24 h earlier. Initially, the calf gained weight at a rate of 0.2 kg per day over the first seven days, but thereafter the gain increased to approximately 2.5 kg per week.

Table I

Creation and outcome of *Camelus dromedarius*, *Lama guanicoe* hybrid pregnancies

Camel sire	Guanaco dam	Sex of the hybrid	Duration of gestation (days)
Musehan	3	Female	291 (aborted)
Musehan	3	Female	365 (stillborn)
Young one/ Musehan	1	-	30 (resorbed)
Young one/ Musehan	4	-	40 (resorbed)
Musehan/ Young one	1	Male	328 (born live)
Musehan	6	Female	302 (aborted)
Guanaco sire	**Camel dam**		
Whalid	660	Female	365 (stillborn)
Whalid	1610	Female	260 (aborted)

Adapted from Skidmore *et al.*, 1999

At 18 months of age the hybrid calf was healthy and vibrant. It exhibited the woolly fiber coat and the nose and nostrils of New World Camelids, but its ears and tail were midway in length between those of the camel and the guanaco. Similarly, its feet were somewhere between the single two-toed conjoined footpad of the camel and the cloven hooves of the guanaco. However, unlike the guanaco, it showed no skin glands on the lateral or medial aspects of the tarsus and there was no sign of the hump that would be present on a camel calf of the same age.

■ DISCUSSION

This is the first report of a successful hybridization between New and Old World Camelids and it was achieved by using artificial insemination and hormone therapy to overcome the marked differences between both parental species in terms of their body size. Although conception rates were low overall it is perhaps significant that the rate was three-fold higher at 15% with fewer attempts (n = 33) when inseminating the smaller number (n = 9) of female guanacos with dromedary semen, than the 4% conception rate achieved when inseminating as many as 30 individual dromedaries on 50 occasions with semen from the single male guanaco. It is possible that some form of undiagnosed subfertility may have existed in this one available male guanaco or he may have been more affected by the heat. However, the work was carried out during the cooler winter months, the semen was of good quality and all the guanacos had been born and bred in the UAE, so should have been acclimatized. It seems therefore much more likely that the marked disparity in conception rates, that has also been reported previously to occur when attempting crosses between other closely related mammalian species, such as horse and donkey (1, 2), sheep and goat (7) and rabbit and hare (3), also existed in our attempts to hybridize New and Old World Camelids.

The apparent female skewing of the sex ratio in the fetuses and neonate (one male:five females) is in accordance with Haldane's law, which states that "when in the F_1 offspring of two different animal species one sex is absent, rare or sterile, that sex is the heterozygous sex" (6). The reason for this skewing may be that the mutation rate of genes of the unpaired segment of the Y chromosome is apparently much higher than that of genes on any other chromosome (11). This is because any genetic defects cannot be repaired by meiotic crossing over with a homologous chromosome. Furthermore, the Y chromosome never enters female germ cells, and germ-line mutations are known to be far more common in the testes than the ovary (11, 12).

The surviving hybrid calf is a male and time will tell if it can produce normal, fertile spermatozoa in the future. If it cannot, it may be that despite the equality in diploid number of chromosomes between New and Old World Camelids (5), a failure of pairing at meiosis prevents formation of normal gametes in the hybrid and thereby prevents the development of a new species which is able to reproduce. This is a pity, since a mixing of some of the physical (body size, hair quality, foot structure) and behavioral (steadfast and stoical versus nervous and flighty) characteristics of both types of Camelid might well produce an interesting and commercially desirable animal.

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Résumé

Skidmore J.A., Billah M., Allen W.R., Short R.V. Utilisation de méthodes de reproduction modernes pour hybrider des camélidés de l'Ancien et du Nouveau Monde : *Camelus dromedarius* x *Lama guanicoe*

Un groupe de neuf femelles et un mâle guanacos a été maintenu au sein d'un important troupeau de dromadaires au Centre de reproduction camelin à Dubaï. Les ovaires des femelles guanacos et dromadaires étaient scannés régulièrement et, quand ils présentaient un follicule dominant de 0,7-0,9 cm de diamètre chez le guanaco ou de 1,3-1,8 cm chez le dromadaire, les femelles recevaient une injection unique de GnRH analogue, buserelin (Receptal ; dromadaires : 20 µg i.v. ; guanacos : 10 µg i.v.) pour induire l'ovulation et subir une insémination 24 h après. Trente femelles dromadaires ont été inséminées à 50 occasions avec 2-4 ml de semence de guanaco diluée à 1:1 avec du tampon vert (150-400 x 10^6 spermatozoïdes motiles), résultant en deux gestations ; l'une a donné un produit mort-né au 365^e jour de gestation et l'autre s'est terminée par un avortement au 260^e jour. De la même manière, neuf femelles guanacos ont été inséminées à 34 occasions avec de la semence diluée de dromadaire (300-400 x 10^6 spermatozoïdes motiles), donnant lieu à six conceptions ; deux ont disparu par résorption entre 25-40 jours après l'ovulation, deux ont abouti à des avortements respectivement aux 291^e et 302^e jours, une autre s'est terminée par un produit mort-né au 365^e jour et la dernière a produit un mâle hybride né vivant au 328^e jour. A la connaissance des auteurs, c'est le premier hybride viable de camélidés du Nouveau et de l'Ancien Monde. Son existence souligne un remarquable degré de conservation des processus de reproduction entre ces deux espèces.

Mots-clés : *Camelus dromedarius* - *Lama guanicoe* - Hybridation - Insémination artificielle - Emirats arabes unis.

Resumen

Skidmore J.A., Billah M., Allen W.R., Short R.V. Uso de métodos reproductivos modernos para hibridizar camélidos del Nuevo y del Viejo Mundo: *Camelus dromedarius* x *Lama guanicoe*

Se mantuvo un grupo de guanacos, nueve hembras y un macho, junto con un hato mayor de dromedarios en el Centro de Reproducción del Camello en Dubai. Los ovarios de las hembras guanaco y de las camellas fueron examinados en forma regular por «scan» y, al presentar un folículo dominante, de un diámetro de 0,7-0,9 cm en el guanaco o de 1,3-1,8 en la camella, se administró una inyección única de GnRH análoga, buserelina (Receptal, camellos 20 µg i.v., guanacos 10 µg i.m.), con el fin de inducir la ovulación, e inseminando 24 h más tarde. Se realizaron 50 inseminaciones en 30 hembras de dromedario, con 2-4 ml de semen de guanaco, diluido 1:1 con buffer verde (150-400 x 10^6 motilidad espermática), resultando en dos preñeces, una de las cuales fue un natimuerto al día 365 de gestación y la otra fue abortada en el día 260. De manera similar, nueve hembras guanaco fueron inseminadas 34 veces, con semen de dromedario diluido (300-400 x 10^6 motilidad espermática), resultando en seis concepciones, dos fueron reabsorbidas entre el día 25-40 post ovulación, dos fueron abortados en los días 291 y 302 respectivamente, una fue natimuerto en el día 365 y un macho nació vivo al día 328. Según los autores este es el primer híbrido viable hasta ahora entre camélidos del Viejo y el Nuevo Mundo y su existencia demuestra un grado importante de conservación de los procesos reproductivos entre ellos.

Palabras clave: *Camelus dromedarius* - *Lama guanicoe* - Hibridación - Inseminación artificial - Emiratos arabes unidos.